Controlled release of aldicarb from carboxymethyl cellulose microspheres: *in vitro* and field applications

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Abstract: Aldicarb is a carbamate pesticide that is widely used throughout the world in the protection of crops (eg cotton, nuts, potatoes, onion, tobacco, sugar beet and sugar cane). In Turkey, especially in the Çukurova region, it is used for the control of the cotton white fly (*Bemisia tabaci*) which attacks cotton plants cultivated in this region. Aldicarb contamination in surface and ground water is a serious problem in several countries, partly due to its high water solubility. It is also highly toxic to mammals. In order to overcome these problems, microspheres of aldicarb were prepared using carboxymethyl cellulose (CMC) as the biodegradable support material cross-linked with aluminium chloride. A strong hysteresis behaviour was observed upon drying and reswelling. Encapsulation efficiency was in the range 12–23% and aldicarb contents of 5.7–10.3 mg per 100 mg of microspheres was achieved. *In vitro* release was distinctly Fickian, and Higuchi constants were very close to 0.5. Release in pots revealed that only one sample had a release capability for more than four weeks. In the cotton plot much longer durations of release (more than seven weeks) were observed while a commercial granular formulation released its content immediately. It was thus possible to construct a controlled pesticide release system that prolonged the bioavailability to about eight weeks.

Keywords: aldicarb; carboxymethyl cellulose; microsphere; controlled release; biodegradable

1 INTRODUCTION

The control of pests often requires periodic application of pesticide to the crop using conventional formulations, eg powders, granules, or concentrated emulsions. These lead to significant levels of environmental pollution due to the application of extensive quantities of pesticide required to prolong effectiveness. Losses can occur due to wash-out, evaporation, surface run-off and dispersion to unintended regions,[1–3] which also has undesirable economical consequences. Such disadvantages can be overcome by providing low levels of the pesticide over the desired period using a bioactive material release system based on the diffusion of the bioactive compound through a matrix or membrane.[4–6]

Various studies have been carried out to control the release of pesticides and pharmaceuticals to achieve the desired results over a prescribed duration.[6–8] Biodegradable matrices are especially preferred in pesticide formulations in order to prevent a different kind of pollution created by residues of depleted, non-degradable pesticide carriers.

Aldicarb is a carbamate pesticide used for the control of a variety of plant pests (nematodes, insects and mites) for the protection of cotton, tobacco, citrus, potato, etc. It has a high mammalian toxicity (acute oral LD_{50} value for rats: 0.84 mg kg^{-1}) and inhibits reversibly acetylcholinesterase activity.[9] The high water solubility of aldicarb (6 g litre^{-1}) has made it a threat to human health due to the risk of drinking water contamination, which is supported by various ground water contamination studies.[10,11] It is thus a good candidate for use in a controlled-release form.[12]

Sodium carboxymethyl cellulose (NaCMC) can be converted into a hydrogel with ease via chelation with polyvalent cations.[13,14] NaCMC also possesses the advantages of being biodegradable, hydrophilic and economic. It was therefore decided to construct a

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Table 1. Preparation conditions of different aldicarb-containing CMC microspheres

Sample code	*CMC*[a] (g litre^{-1})	*AlCl$_3$* (M)	*Aldicarb (g)/ CMC solution (cm^3)*
L46	40 (LO)	0.6	1.0/50
H22	20 (HI)	0.2	0.5/50
H24	20 (HI)	0.4	0.5/50

[a] HI and LO indicate high- and low-viscosity CMC, respectively.

controlled-release system in which NaCMC would serve as the matrix material for the controlled release of aldicarb.

2. EXPERIMENTAL

2.1. Chemicals

Carboxymethyl cellulose sodium salt (NaCMC), low-viscosity (purity > 99.5%, viscosity of 40 g litre^{-1} aqueous solution at 25 °C: 90–200 mPa s) was obtained from Fluka, AG (Switzerland) and high-viscosity (viscosity of 10 g litre^{-1} aqueous solution at 25 °C: 1500–3000 mPa s) were purchased from Sigma Chemical Company (USA).

Aluminum chloride (anhydrous) and methanol (HPLC grade) were purchased from Merck AG (Germany).

Aldicarb [2-methyl-2-(methylthio)propionaldehyde *O*-methylcarbamoyloxime], was extracted from a commercial 150 g kg^{-1} granular formulation (Temik®; Rhone Poulenc, Turkey) and purified by crystallization from diethyl ether. The purified aldicarb was stored at 4 °C until use.

2.2 Preparation of carboxymethyl cellulose and carboxymethyl cellulose-aldicarb microspheres

Carboxymethyl cellulose (CMC) and CMC-aldicarb microspheres were prepared by cross-linking with aluminum chloride. Aqueous solutions of high-viscosity CMC (HI-CMC) (20 g litre^{-1}), low-viscosity CMC (LO-CMC) (40 g litre^{-1}) and aluminum chloride in the concentration range of 0.2–0.6 M were prepared in distilled water.

For the preparation of CMC-aldicarb microspheres, finely ground aldicarb crystals (sieved through Millipore stainless steel filter, pore size: 0.25 mm) were mixed with the CMC solution. The compositions of the CMC-aldicarb formulations are presented in Table 1. CMC solution or CMC-aldicarb suspension (100 cm^3) was introduced into aluminum chloride solution (1000 cm^3) with a peristaltic pump (Scientific Industries Inc, USA) at a flow rate of 120 cm^3 h^{-1}, through a nozzle (0.2 mm needle inner diameter), and a coaxial airstream was used to control the size and the shape of the CMC drop and to prevent tailing.[15] Aggregation of the CMC drops during the curing period was prevented by continuously stirring the cross-linking solution at a rate of 385 rev min^{-1}. The microspheres formed were filtered, washed twice with 200 cm^3 of distilled water, and dried at room temperature.

2.3 Detection of aldicarb by HPLC

The absorption spectrum of aldicarb was obtained with a Shimadzu UV-2100S (Japan) spectrophotometer. An isocratic reverse-phase HPLC separation system combined with multiple UV detection (Shimadzu C-R4A) was used for the analysis of aldicarb and aldicarb oxime. The mobile phase, methanol + water (40 + 60 by volume), was pumped with a flow rate of 1 cm^3 min^{-1} to the cyanopropyl-silica HPLC separation column (6 mm ID. × 15 cm length, Shimadzu Shimpack CLC series).[16] Before injection of the sample, the column was equilibrated by degassing the mobile phase in a Knauer Degasser Unit and passing it through the column until the baseline stabilized. Initially, all the solutions were screened for the presence of interfering substances and aldicarb and its derivatives were then analysed and compared in terms of their retention time.

Aldicarb standards in distilled water were prepared and their standard curves were plotted as area of the peak vs concentration. The amount of aldicarb was determined by direct injection of a 20 µl aliquot of the sample solutions.

2.4 Characterization of CMC microspheres

2.4.1 Determination of swelling and hysteresis behaviours

Dry CMC microspheres (5 g) were placed in distilled water. At 5-min intervals, the solvent was decanted, traces of water were removed by blotting with filter paper, the spheres were weighed and then returned to the water. This was continued till equilibrium was achieved.

After a final water removal by blotting, they were placed in a vacuum oven (50 °C) and dried and weighed. This hydration-dehydration cycle was repeated three times to determine the hysteresis behaviour of CMC microspheres. Swelling was expressed based on the water absorbed by the microspheres as:

$$\text{Swelling}(\%) = ((W_s - W_d)/W_d) \times 100 \qquad (1)$$

where W_d is the weight of the dry microspheres and W_s is the weight of swollen microspheres

2.4.2 Determination of encapsulation efficiency of aldicarb

CMC-aldicarb microspheres (75 mg) were homogenized in distilled water (2 cm^3) in a glass-glass homogenizer, diluted 15 times with distilled water in a flask and stirred continuously with a magnetic stirrer overnight. An aliquot of that solution (1.5 cm^3) was transferred into an Eppendorf centrifuge (model: 5415C) and centrifuged (14 000 rev min^{-1}; 10 min). The supernatant was analysed spectrophotometrically at 245 nm for the determination of aldicarb encapsulation efficiency of CMC microspheres using a calibration curve constructed with distilled water as the solvent. The results were presented as (i) aldicarb

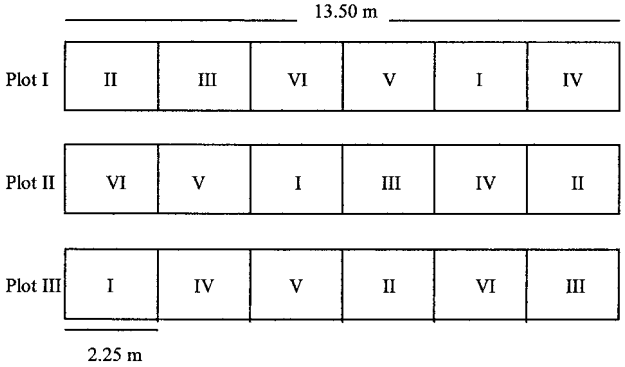


Figure 1. Schematic presentation of the experimental plot study. Patch I. Control (Plant +), Patch II. Control (Plant −), Patch III. H22, Patch IV. H26, Patch V. L46, Patch VI. Temik (Plots I, II and III contain the same applications but the respective positions of the patches are different. Each patch consisted of three rows of plants/formulations).

content, which was defined as amount of aldicarb (mg) per 100 mg of microspheres, and (ii) as encapsulation efficiency, which was defined as aldicarb in microspheres (mg) as a percentage of aldicarb input (mg).

2.4.3 Scanning electron microscopy (SEM)

CMC and CMC-aldicarb microspheres were coated with gold under vacuum and their scanning electron micrographs were obtained using a Jeol (JSM-6400) scanning electron microscope.

2.5 Release studies

2.5.1 In vitro continuous release study

Aldicarb-loaded CMC microspheres (200 mg) were placed in a continuous flow cell (8 cm^3) in the experimental set-up reported earlier.[17] Distilled water was introduced through the bottom inlet port of the flow cell at a constant rate (80 $cm^3 h^{-1}$) at 25 °C. The effluent was collected at 1-h intervals and the released aldicarb was determined spectrophotometrically at 245 nm.

2.5.2 Aldicarb release in pots

Twenty-one cotton seeds (*Gossypium hirsutum* L) were sown in each pot (40 cm height × 40 cm diameter) which were filled with equal amounts of soil. Excess plants were removed leaving seven plants per pot. Five types of formulation were applied: H22, H24, L46 (see Table 1), Temik and control (no microspheres or Temik). All the applications were carried out in duplicate.

Samples from each formulation (70 mg of microspheres) were weighed and packaged in a 10 × 10 cm gauze pouch. Each gauze pouch was placed 5 cm below the surface at the root of one cotton plant in the pot. The pots were irrigated at a rate of 7 litres per $week^{-1}$ for a period of seven weeks. Formulation contained in pouches and surrounding soil was collected each week for seven weeks to carry out the analytical procedures.

In order to determine the released aldicarb, soil (10 g) was put in a Nalgene centrifuge tube (50 cm^3) and hydrated with water (3 cm^3). After 30 min, methanol (15 cm^3) was added and the tubes were mixed vigorously with a whirl shaker for 5 min and then shaken with an orbital shaker overnight. The soil samples were centrifuged (4500 rev min^{-1}; 15 min) and the supernatant was analysed by HPLC according to Section 2.3, using methanol + water (40 + 60 by volume) as the mobile phase.

The microspheres collected from the soil were washed with distilled water (10 cm^3) to prevent interference due to ingredients in the soil. The aldicarb remaining in the CMC microspheres was extracted as described in Section 2.4.2 and analysed in a UV spectrophotometer (Shimadzu UV-2100S, Japan) at 245 nm.

2.5.3 Aldicarb release in an experimental cotton plot

Plots were 13.5 m long, consisting of six patches of 2.25 × 6 m^2 area. Each patch had three rows and each row contained 30 cotton plants (Fig 1).

Soil was fertilized with 12 kg $decare^{-1}$ nitrogen and 6 kg $decare^{-1}$ phosphorus. Each formulation was weighed to provide the desired amount of pesticide (30 mg aldicarb per plant) and packaged in gauze pouches. Sample application, collection and analysis were carried out according to the procedures given in the previous section.

3 RESULTS AND DISCUSSION

In this study, carboxymethyl cellulose microspheres cross-linked via aluminum ions were used as a matrix material for aldicarb release. The aim was to minimize the aldicarb in the environment, by reducing its rate of release.

Characterization of CMC microspheres was realized (eg by determining their water content, aldicarb content, encapsulation efficiency, etc) and release behaviour was studied *in vitro*, in pots and in the cotton fields.

3.1 Detection of aldicarb by HPLC

Isocratic separation of aldicarb and its derivatives using purely alkyl bonded phases has been unsuccessful because the high organic modifier contents necessary to elute the relatively non-polar parent compound aldicarb within a reasonable time resulted in an inadequate separation of aldicarb oxime and aldicarb sulfone.[18–20] It was shown that interactions with phenyl and cyano phases are weaker and, indeed, aldicarb can be eluted from these columns efficiently in water-rich mobile phases. In addition, to get a successful separation of these compounds, the effect of organic modifier content was analysed with different columns by Lin and Cooper and it was found that phenyl/water-methanol, cyano/water-methanol and cyano/water-acetonitrile bonded and mobile phase pairs gave acceptable separations.[20,21] Therefore, cyano/water-methanol pair with a 60 + 40 (by volume) water + methanol ratio, was used for HPLC analysis of

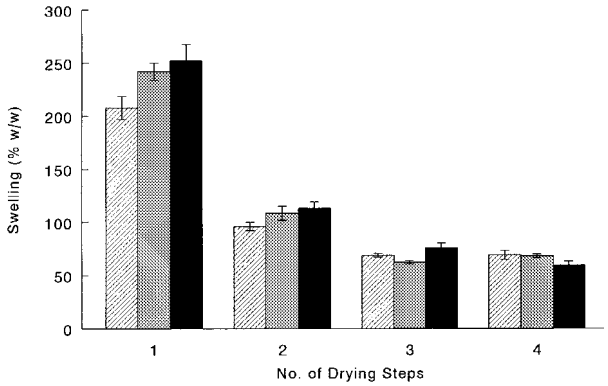


Figure 2. Swelling of (▨) H22, (▤) H24 and (■) L46 formulations after repeated drying steps.

aldicarb and its derivative, aldicarb oxime (if any formed).

HPLC calibration curves were constructed both at 214 nm and 245 nm by plotting peak area vs concentration. Although 245 nm was preferred for the analysis of aldicarb alone, to detect both aldicarb and aldicarb oxime in the same run, 214 nm was used.

3.2 Characterization of microspheres

3.2.1 Hysteresis behaviour and swelling time of CMC microspheres

The effect of multiple hydration-dehydration cycles on swelling behaviour of CMC microspheres was analysed and results are presented in Fig 2.

It had been expected that the swelling value obtained for L46 would be lower than that of the formulations prepared with high-viscosity CMC, due to the higher density of polymer fibres in the microsphere structure. However, it had almost the same level of water content as the other formulations, most probably because, although the chains were more numerous, they were much shorter. There was a surprising result showing that H24 swelled more than H22. It was expected that lower swelling values would be obtained as the cross-linker concentration increased. Here, there is a dual effect by $AlCl_3$; it decreases the pH of the solution by the formation of hydrochloric acid and it is also the cross-linking agent.

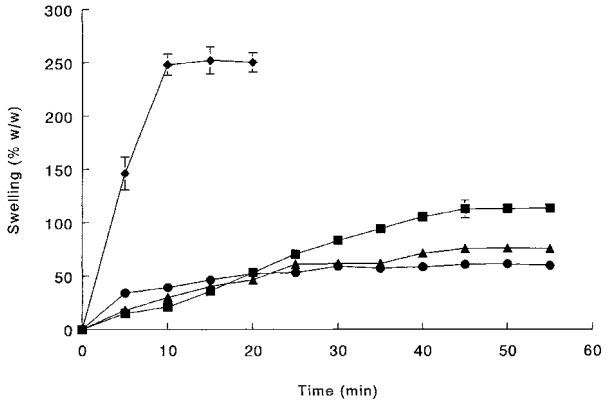


Figure 3. Swelling of formulation L46 after (◆) first, (■) second, (▲) third and (●) fourth drying steps.

Table 2. Aldicarb in CMC microspheres and Higuchi constants

Sample code	*Aldicarb content*[a]	*Encapsulation efficiency (%)*	k_H $(h^{-1/2})$[b]
L46	10.3 (±0.71)	23.0 (±1.78)	0.524
H22	5.7 (±1.08)	12.1 (±2.44)	0.535
H24	9.1 (±0.84)	19.9 (±2.04)	0.565

[a] Milligrams aldicarb per 100 mg microspheres.

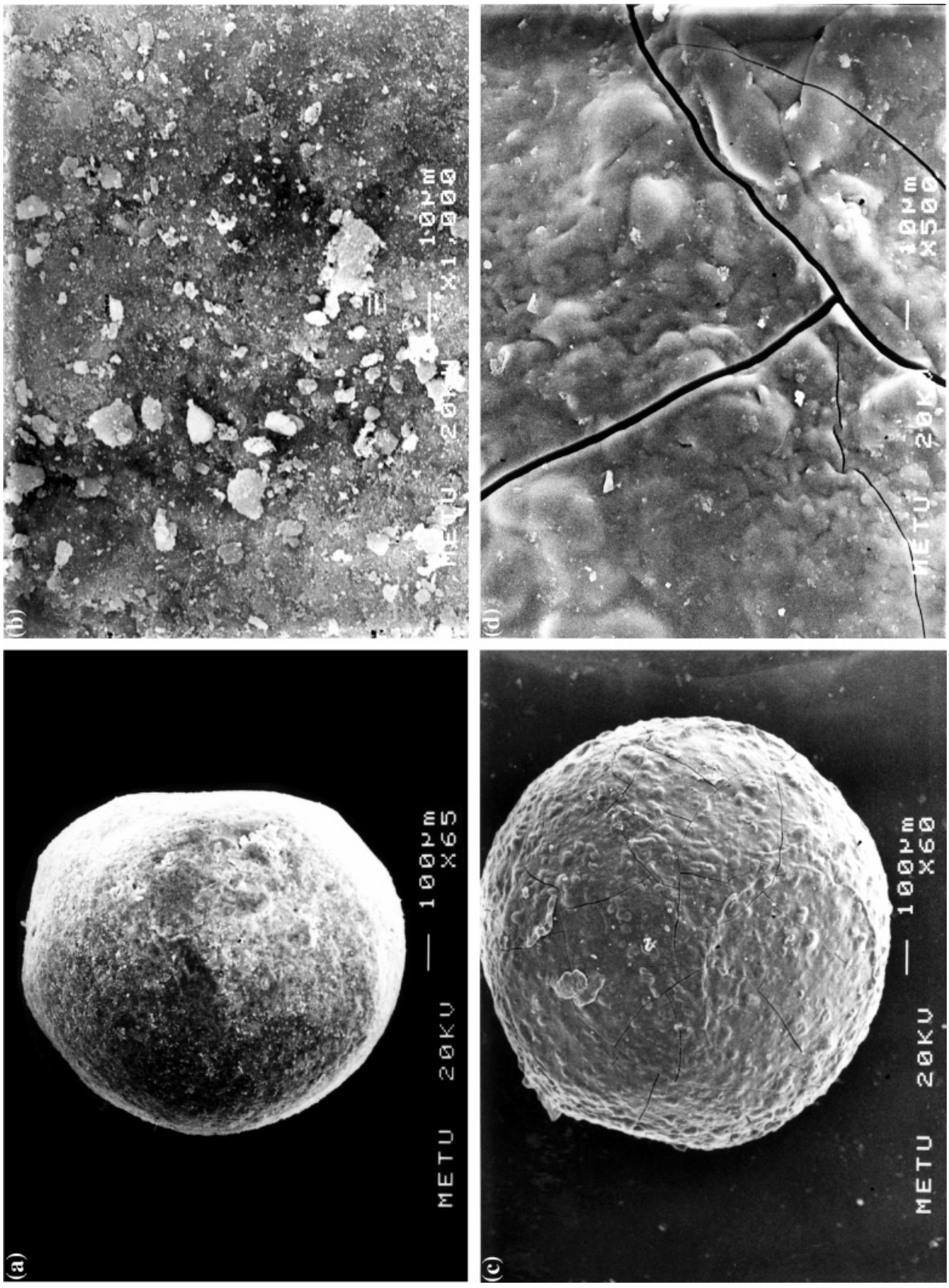
[b] Higuchi constants (k_H): slope of the fraction released vs $t^{1/2}$ plots.

The change in pH affects the COO^-/COOH ratio on the CMC and thus the conformation of the CMC molecules and the number of available sites (COO^- groups) for cross-linking. In other words, increasing the $AlCl_3$ concentration yields a more acidic solution and this leads to the protonation of the carboxylic groups and thus to a decrease in the cross-linkable sites. All these would produce a loosely cross-linked structure.

L46 reached a maximum swelling value (after the first cycle) of about 250% (w/w) within 10 min, while after the second and third drying periods maximum swelling values of about 98% and 85% (w/w) were reached only after about 40 min, respectively (Fig 3). Thus, the swelling capacity of the CMC microspheres decreased and the time to equilibrium swelling increased after each drying step and reached a constant value after the third drying. This observation was also reported by Pussemier and Debongnie[22] for alginate microspheres prepared for the controlled release of aldicarb and carbofuran. This hysteresis phenomenon is probably due to the formation of additional intermolecular bonds (hydrogen bonds and ionic bonds) during drying, which are not destroyed during rehydration. Rapid water removal during dehydration favours the association of carboxyl groups with hydrogen and this leads to the formation of hydrogen bonds. In addition, it has been shown that only a fraction of the carboxyl groups participate in the initial formation of strong intermolecular bonds in hydrated gels.[23] Thus, drying causes the formation of additional, probably less stable, bonds which irreversibly decrease the extent and rate of water uptake.

3.2.2 Encapsulation efficiency and aldicarb content

Encapsulation efficiency was defined as the aldicarb found in the microspheres (mg) as a percentage of the input aldicarb (mg). Aldicarb content was defined as the amount of aldicarb present (mg) in 100 mg of microspheres. The encapsulation efficiency and aldicarb content of aldicarb-CMC microspheres are given in Table 2. The results show that encapsulation efficiency and aldicarb contents are quite low. This is a result of the high water solubility of aldicarb (6 g litre^{-1}) which causes aldicarb to leach out while in a continuous medium during cross-linking. In another study carried out under identical conditions but with a much less soluble compound (diclofenac sodium), the encapsulation efficiency was found to be


(a)
METU 20KV —— 100µm X65
(b)
METU 20KV —— 10µm X1,000
(c)
METU 20KV —— 100µm X60
(d)
METU 20KV —— 10µm X500

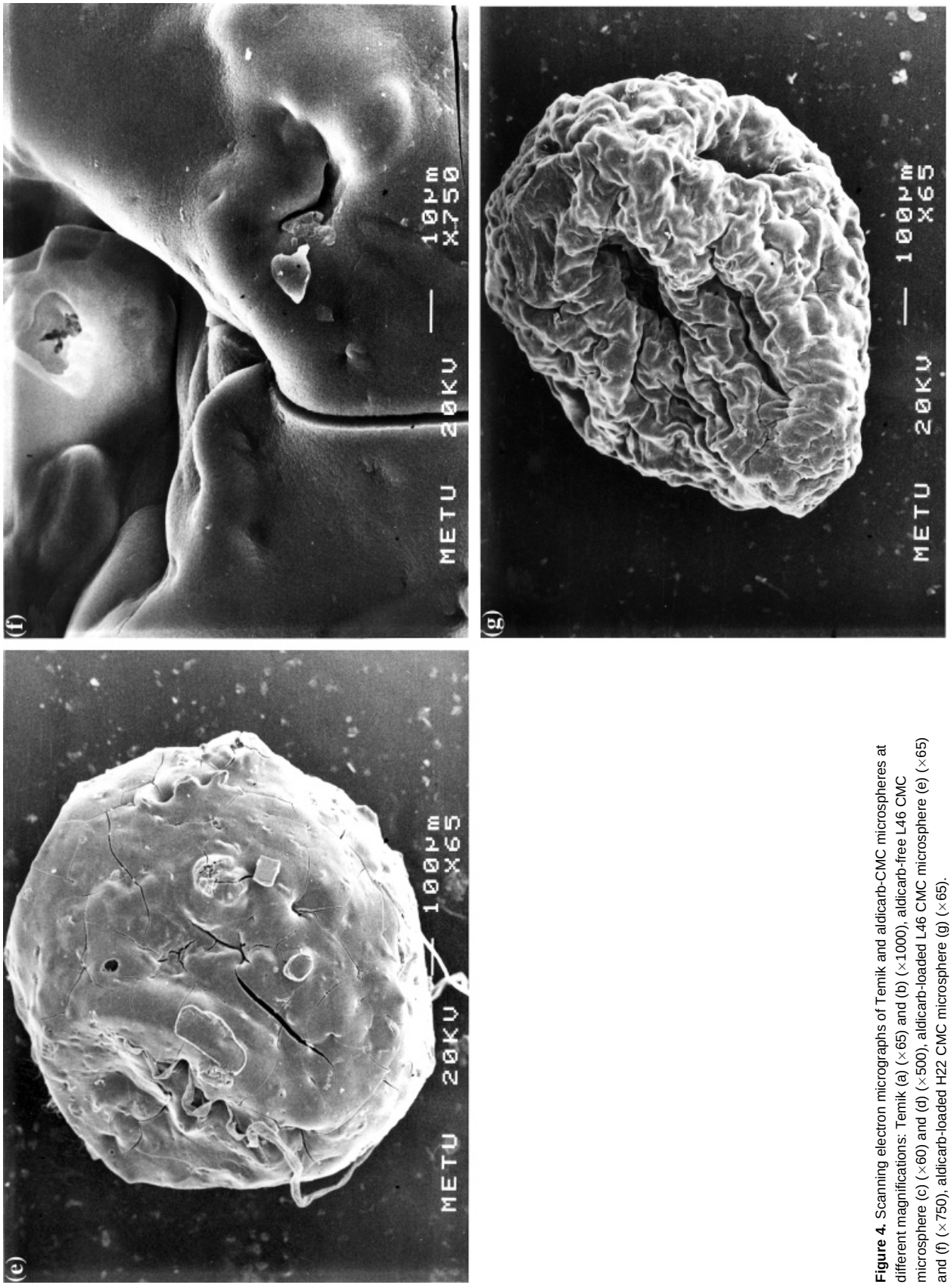


Figure 4. Scanning electron micrographs of Temik and aldicarb-CMC microspheres at different magnifications: Temik (a) (×65) and (b) (×1000), aldicarb-free L46 CMC microsphere (c) (×60) and (d) (×500), aldicarb-loaded L46 CMC microsphere (e) (×65) and (f) (×750), aldicarb-loaded H22 CMC microsphere (g) (×65).

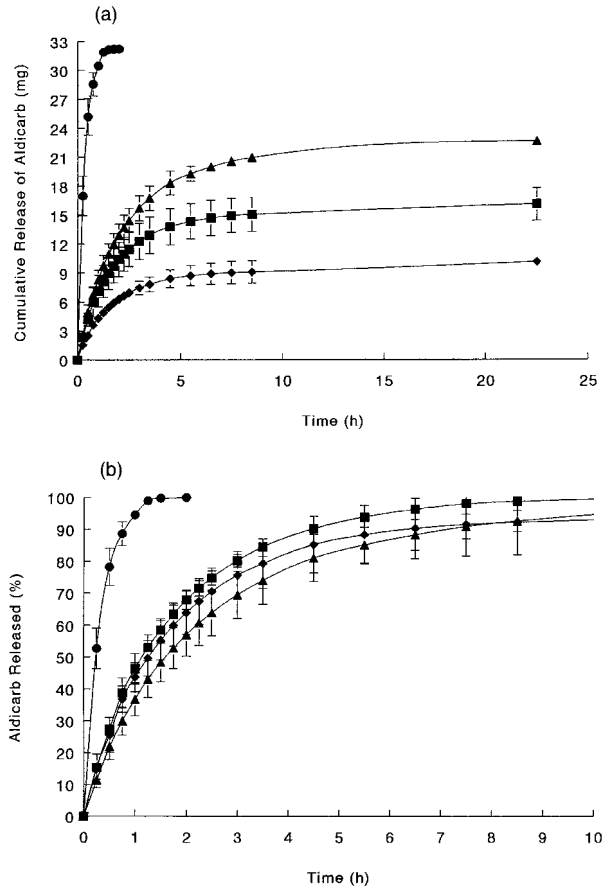


Figure 5. *In vitro* release of aldicarb from (●) Temik, (◆) H22, (■) H24 and (▲) L46 in distilled water presented as (a) cumulative release and (b) percentage release vs time.

48–89% which is about four times greater than that obtained with aldicarb.[7]

Because of this problem, the decision was taken to saturate the continuous medium with aldicarb (and to decrease the concentration gradient) in later applications.

3.2.3 Scanning electron microscopy

SEM micrographs of the Temik, CMC and CMC-aldicarb microspheres are presented in Fig 4(a)–(g).

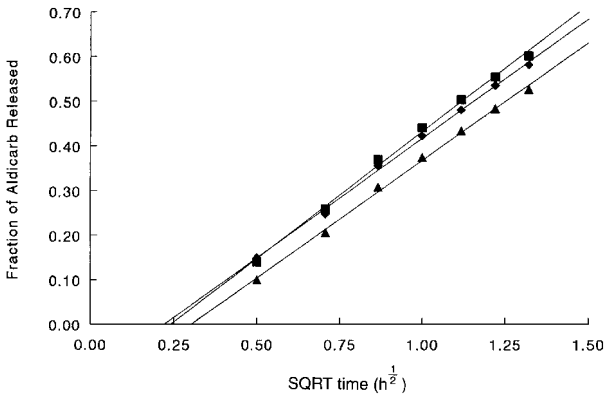


Figure 6. *In vitro* release of aldicarb from (◆) H22, (■) H24 and (▲) L46 in distilled water plotted according to Higuchi.

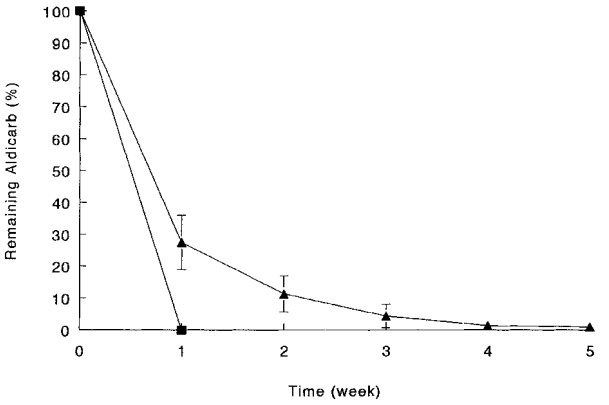


Figure 7. Loss of aldicarb from (▲) L46 and (■) Temik, H22 and H24 formulations in pot experiments.

The microspheres had quite a uniform size distribution, with an average diameter of 1.25 mm and a low standard deviation (±0.08). The commercial granular formulation of aldicarb, Temik (Fig 4(a),(b)), appears to consist of very fine particles and does not have the appearance of the CMC microspheres (Fig 4(c)–(g)). This compacted powder appearance is in accordance with its rapid disintegration in water. Figure 4(c) shows an aldicarb-free L46 CMC microsphere that is a perfectly spherical particle. An aldicarb-loaded L46 microsphere appeared to have the same dimensions as the unloaded one, but it was not as spherical as the other, probably due to the aldicarb crystals in its structure (Fig 4(e)). The appearance of loaded H22 is quite striking (Fig 4(g)). This is evidence that it is the polymeric continuous phase that prevents the microsphere from fragmenting when exposed to water. That there is no similar collapse with L46 is due to the high CMC and high cross-linker of this formulation.

3.3 Release studies

3.3.1 In vitro continuous release of aldicarb

Diffusion-controlled matrix systems where the active agent is uniformly dissolved or dispersed in a polymer matrix generally exhibit release behaviour starting with a substantial release which gradually diminishes with time.[24] This was found to be true for the CMC-aldicarb microspheres, too (Fig 5(a)). The release trend was almost identical for the three formulations (H22, H24 and L46) and distinctly different from that of Temik (Fig 5(b)).

Several methods can be used for the theoretical treatment of similar data and to obtain a quantitative representation of release. One of these methods, presented by Higuchi[25] for release controlled by diffusion and swelling, predicts that the cumulative percentage released would be proportional to the square root of time. So, when the fraction of the pesticide released was plotted against $t^{1/2}$ for the three formulations, a straight line was obtained up to 55% release with slopes varying between 0.524 and 0.565 (Fig 6; Table 2). This indicated that the release kinetics are consistent with a dispersed monolithic-

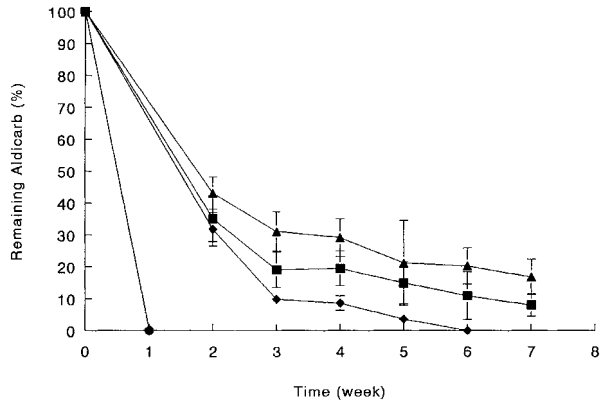


Figure 8. Loss of aldicarb from (●) Temik, (◆) H22, (■) H24 and (▲) L46 formulations in experimental cotton plots.

type release system. This was quite expected because the pesticide was highly water-soluble, the microspheres swelled up very rapidly so, except for a slight influence of the matrix, the major factor controlling release was the aldicarb content of the microspheres. The slope was found to be 1.095 for Temik so it can be said that the release rate was substantially reduced by this controlled-release system.

3.3.2 Release in pots

The release of aldicarb from CMC microspheres (H22, H24 and L46) was also tested in pots containing planted cotton. As can be seen from Fig 7, release beyond one week could only be observed with L46 microspheres. It can be seen that the aldicarb release from L46 continued for three to four weeks under high irrigation conditions. This is significantly longer than it was *in vitro*. No aldicarb could be detected in the soil samples collected from the pots around the sites where the three formulations were embedded. Minute amounts of aldicarb could be detected only in the pot treated with Temik but in this case there was no trace of Temik granules. It can be concluded that the formulation L46 developed in this study had achieved the goal, probably due to its higher aldicarb content, and released the aldicarb for long periods with low rates.

3.3.3 Release in the experimental cotton plot

For each formulation (H22, H24 and L46), the amount of residual aldicarb was determined by extracting the microspheres taken from six different pouches. The average amount of aldicarb detected in the formulations for seven weeks was used to construct Fig 8. The results showed that there was still a considerable amount of aldicarb left in L46 (16.8%) and H24 (8.0%) microspheres at the end of seven weeks, whereas there was no aldicarb left in the H22 formulation. This length of time is apparently much longer than *in vitro* and in pots and is definitely longer than with Temik.

In the collected soil, however, no aldicarb could be detected in any of the samples and in the commercial Temik formulation. Since the soil was collected from 5 cm below the surface, aldicarb could have migrated deeper in the soil and thus not detected. This rapid downward movement of aldicarb could be due to preferential flow, which is the rapid movement of chemicals through a small fraction of the soil volume such as cracks or fissures (Pers comm, Flury *et al*, CiGa, 1994). It can also be due to biodegradative activity of soil micro-organisms which are known to take part in the bioremediation process.

The influence of aldicarb-containing formulations, in comparison to Temik and the control, on the crop yield, plant height, fibre thickness and strength of the cotton plant was of little significance. This was possibly due to the plot not being attacked by the white fly during the course of the test. Further and repeated trials to show the true worth of the formulations needs to be carried out.

4 CONCLUSION

The aim of the study was to prepare formulations containing aldicarb which would have significantly lower rates of pesticide release than the commercially available granule. This would enable smaller quantities of pesticide to be used over a longer duration, with lower run-off. The biodegradable matrix material used here, CMC, probably due the hydrophilic nature of both it and aldicarb, did not create a significant resistance to the diffusion of the solute. Thus the difference between the supports prepared with different concentrations of cross-linker, or with CMC of different properties, was not revealed in their release behaviour *in vitro*. The trend was identical for all the microsphere types, and distinctly different from that of the commercial granule, Temik. In the pots, only L46 could be detected because of the use of equal amounts of microspheres that had different loadings so that the aldicarb contained in H22 and H24 was too low for detection.

However, the difference between the microsphere types became distinct when applied to experimental plots. Here, the main criterion was to apply a sufficient amount of microspheres to contain a total of 30 mg of aldicarb per pouch. Thus the microsphere weights used were different. In this case, release was most rapid with Temik, followed by H22, H24 and L46, in that order. The basic reason for this appears to be that L46 and H24, with their higher encapsulation efficiencies, carried more aldicarb per unit microsphere, and that dissolution and diffusion of aldicarb from the low-aldicarb-containing H22 was faster. In this application the influence of encapsulation efficiency became obvious.

In conclusion, it was possible to construct aldicarb-containing CMC microspheres that prolonged the availability of the pesticide through controlled release. These results hold out the hope that pollution caused by excessive application of the commercial formulations of aldicarb may be significantly decreased.

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